PREPARATION OF HYBRID SCAFFOLD BASED ON PVA AND BIOACTIVE GLASS CERAMICS

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Submitted by:

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CERTIFICATE

This is certified that the work contained in the project entitled "**PREPARATION OF HYBRID SCAFFOLD BASED ON PVA AND BIOACTIVE GLASS CERAMICS**" by Soumya Sourav (Roll 110CR0616) in partial fulfilment of the requirements of the award of Bachelor of Technology Degree in Ceramic Engineering at the National Institute of Technology, Rourkela is an authentic work carried out by him under my supervision and guidance.

To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other university / institute for the award of any Degree or Diploma.

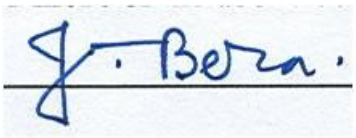

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FIGURE LIST

TABLE LIST

ABSTRACT:

A hybrid scaffold based on polyvinyl alcohol and bioactive glass ceramics was synthesized and characterized for its biocompatibility and its degradation behavior. Poly vinyl alcohol and glass ceramics were mixed in the ratio 70:30 by weight percentage. The glass ceramic was based on 65 % SiO_2, 5 % P_2O_5 and 30 % CaO. Aqueous solution of PVA was prepared with 25% concentration. A glass ceramic sol containing TEOS, $CaCl_2$, TEP was added to PVA solution. The pH of the final solution was adjusted to 2 using HF. Hybrid gel formed was then dried and characterized for phase evaluation by XRD, chemical characterization by FTIR, AP, BD, microstructure by FESEM, biodegradability and bioactivity. XRD pattern revealed that the hybrid was amorphous material. SEM microstructure showed crystallization of glass ceramic phase in polymer matrix. Incubation of glass ceramic in SBF solution showed the deposition of Calcium and Sodium containing phases on the surface of scaffold. This indicates that the hybrid scaffold is biocompatible and may be applicable for uses in bone tissues engineering.

CONTENTS

1. INTRODUCTION

There is a an ever rising requirement for the growth of novel and progressive things for repairing of bone tissue, mainly due to swelling life expectancy. Essentially, the goal of tissue engineering is to reinstate impaired tissue to its functional state, reducing the requisite for replacements in joints and transplants. The encounters in tissue engineering are related with the development of proper scaffold materials that can act as models for cell propagation [1–2].To be precise, bone tissue engineering includes biodegradable 3D scaffolds to patch-up bone tissues. The scaffolds ought to have a satisfactory pore size and interconnectivity in order to stimulate cell evolution, decent biocompatibility, and meticulous biodegradation kinetics thereby providing a preliminary biomechanical backing until cells produce the extracellular matrix. The scaffold is unceasingly degraded as well as metabolized during the formation and association of matrix,. Accordingly, the degradation behavior of the scaffold formed is a disparagingly vital obligation in tissue engineering applications [2–7]. A diversity of biomaterials has been developed as synthetically manufactured scaffolds that can kindle the three dimensional tissue evolutions [8]. In the midst of them, related biomaterials have been frequently studied for bone tissue engineering owing due to their acknowledged osteoinductive along with the osteoconductive properties [9–12]. Moreover, the dissolution products generated from the bioactive glass govern over genetic factors of bone development [13]. The BG fibers have also been discovered to display in vitro bioactivity and impact cell evolution in culture. The diameters of fibres along with their porosity and the arrangement between them can be designed, making them attractable for usage as resources for implants in tissue engineering. A diminution in the fiber gap has been shown to influence in improved cell proliferation. Fine-tuning of the porosity can thus be adjusted to allow

adequate influx of blood vessels, blood cells and other metabolic exchanges while at the same time permitting for a great population of cells to infiltrate and assimilate within the implant.

Table (1): Composition of 45S5 bioglass and bioglass scaffolds.

(Ref *: J. D. Kretlow and A. G. Mikos, "From material to tissue: biomaterial development, scaffold fabrication, and tissue engineering," AIChE Journal, vol. 54, no. 12, pp. 3048–3067,2008.* [1])

Composition (in weight %)	**Bioglass 45S5**	**Bioglass Scaffold**
P	5	26.4
Si	45	21.7
Na	25	10.6
Ca	25	4.3

Table (2): Bioactivity index for bioceramic materials.

(Ref: J. D. Kretlow and A. G. Mikos, "From material to tissue: biomaterial development, scaffold fabrication, and tissue engineering," AIChE Journal, vol. 54, no. 12, pp. 3048–3067, 2008. [1])

	I_B value
45S5 Bioglass	12.6
Ceravital	5.7
Glass ceramic	3.3
HA(hydroxyapatite)	3.1
Alumina	0

As stated by *P. Fabbri, V. Cannillo, A. Sola, A. Dorigato, and F. Chiellini* [3], we came to know that one of the most important parameters to measure bioactivity is the bioactivity index (I_B).

$I_B = 100 / t_{O.5bb}$

Here $t_{0.5bb}$ is considered as the time taken to form half of the bonding zone (interface between bone and implant).

NOTE: The greater the I_B value, the lower is the shear strength of that material.

In general, there are two types of bioactivity [3]:

- Class A: Here biological surface is colonized by osteogenic stem cells. Both extra and intra cellular is generated at the interface. Materials showing this type of bioactivity are both osteo-conductive and osteo-inductive. Example: Bio glass.
- Class B: In this case a bio-conductive pathway allows bone to grow along it, the material exhibiting extracellular response at interface only. These are osteo-inductive only. Example : Hydroxyapatite[$Ca_{10}(PO_{4)6}(OH)_2$]

Still, bioactive glasses, show low mechanical properties in porous forms in comparison to cortical and cancellous bones [6, 10].They have comparatively low fracture toughness, low fracture strength (40-60MPa) and low elastic modulus (30-40MPa).Thus the fabrication of inorganic-organic composites is aimed at creating a balance between toughness, strength and other requisite properties [14]. These composites are carried out via a hybridization route, in which two or more organic and inorganic components are pooled [15]. Chemical crosslinking is a flexible method to adjust and improve the properties of organic-inorganic hybrids as one

may be able to modify their degradation behavior along with physical, biological and mechanical properties [16]. Different ions play different roles in bio activity and regulate bodily functions. Osteoblasts are mono-nucleated cells responsible for production and mineralization of bone matrix. They synthesize collagen, glycoproteins and regulate Ca-P levels. Osteoclasts are multinucleated cells responsible for resorption and breaking down of bone tissue [17].

2. LITERATURE REVIEW

There are mainly two methods to manufacture glass one being the traditional melt-quenching method and the other is the sol–gel method. 45S5 Bioglass are manufactured by melt-quenching method in which oxides are reacted with each other and subjected to melting at temperatures above 1300 ^{o}C in a crucible made up of platinum. This is followed by the quenching process in H_2O with the assistance of a graphite mold. The sol–gel route involves the formation of silica nanoparticles carried out at room temperature. In this method precursors undergo polymer type reactions resulting in gel formation. [18, 19]. The gel is basically a network of covalently bonded wet inorganic silica, which is subjected to drying and heating at around 600 ^{o}C for glass formation. The sol–gel glasses have an inherent mesoporosity compared to glasses formed by melt quenching process. On the other hand melt-quenched glasses have been found to possess comparatively high density [20-22]. The nanoporosity results in enhanced cell proliferation owing due to improved nanotopography [23, 24]. This is due to an augment in specific surface area compared to melt-derived glass having the same compositions [25]. The high surface area of sol–gel glasses gives rise to considerably higher rates of dissolution. In addition to that, sodium is not essential in the composition as there is no melting is involved. The sol–gel process has immense flexibility. Bioactive glasses can be manufactured as nanoporous powders or as monoliths. This can be achieved by manipulating the pH involved during the manufacturing process [26-31]. Example: Tetraethyl orthosilicate (TEOS) i.e ($SiOC_2H_5$) undergoes hydrolysis to form a sol containing nanoparticles under both acidic and basic conditions. Under basic conditions [32], sub micrometer particles and spherical bioactive nanoparticles can be formed. One of the disadvantages of sol–gel is that it is difficult to attain bioactive glass monoliths in the period of drying without the formation of cracks. There are two primary causes of cracking [32]:

- Shrinkage that occurs during the process of drying.
- Evaporation of liquid by-products in the course of condensation reaction.

As vapor is removed from the gels through the interconnected network of pores, it travels from within the gel to the surface. This results in the creation of capillary stresses giving rise to cracking [33-38].

As per *Hench LL.* [39], the reaction kinetics in bio glass i.e the steps involved in hydroxycarbonate apatite formation are:

1. Leeching: Na^+ leeches from bio glass and is substituted with protons or hydronium ions from the solution. The mechanism involved here is controlled by diffusion .The reaction rate is inversely proportional to the square root of temperature. This leeching phenomenon increases pH.

   $Si\text{-}O^-Na^+ + H^+ + OH^- \rightarrow Si\text{-}OH^+ + Na^+(aq) + OH^-$

2. Network Dissolution: It is also referred to as hydrolysis (dissolution of network of bioglass solution) through OH^- ions. The siloxane bonds form a silica rich layer by poly-condensation of neighboring silanols. This mechanism is regulated by an interfacial reaction. The reaction rate is linearly dependent on temperature. The silica rich layer initiates the movement of Ca^{2+} and PO_4^{3-} from bio glass bulk to the surface.

   $Si\text{-}O\text{-}Si + H_2O \rightarrow Si\text{-}OH + OH\text{-}Si$

(NOTE: The release of cations from network of bioglass and formation of both Si and Ca-P layer is possible only if pH = 8.If pH < 8 then no Ca-P precipitation is observed. If pH > 8 then

immediate precipitate of CaP after bio glass immersion in aqueous setting occurs preventing further ion release.)

3. Precipitation: Amorphous Ca-P rich layer is formed by immigration of Ca^{2+} and PO_3^{4-} groups from the glass bulk to its surface by the amalgamation of soluble Ca and P into solution. Crystallization of Ca-P layer occurs by integration of OH^-, CO_3^{2-}, F^- ions to form a hydroxyl, carbonate or flouroapatite layer. [38, 39]

As reported by *S. Verrier, J. J. Blaker, V. Maquet, L. L. Hench, and A. R. Boccaccini,* [15], in-vitro studies using sol-gel derived bio glass foams exhibited that these scaffolds could stimulate primary human osteoblasts to yield mineralized bone nodules without the requirement for osteogenic supplements. In a sol-gel process inorganic networks are developed through establishment of a colloidal suspension followed by its subsequent gelation. The colloid synthesis requires precursors which are most likely a metal/metalloid element bounded by many reactive ligands. Among sol-gel precursors metal alkoxides/alkoxysilanes are the most suitable as their reaction with water is voluntary. [15–18].

From the ref. *T. Yamaoka,* [19], Polyvinyl alcohol (PVA) has been found an implant material in numerous biomedical applications such as drug delivery systems, surgical repairs and artificial skin primarily due to its suitable attibutes. It has considerably high mechanical strength, nontoxicity, and biocompatibility [19, 20]. Other factors like the degree of hydrolysis affects its physical-chemical properties like pH-sensitivity, viscosity, hydrophilic/hydrophobic exchanges and solubility. Furthermore, water and alkoxides are not miscible. This gives rise to the requirement of a mutual solvent which can be used as a homogenizing agent in order to aid hydrolysis. Catalysts can also be used to further diminish this miscibility gap. Sol of the glass is

mixed with PVA of different % compositions to gel the hybrid nd scaffold preparation is carried out either through surfactant assisted foaming method or sacrificial template method. [20]

As reported by *Swati Midha, et al.* [29], the primary requisites for bioactive scaffold material : Interconnected macro porous network with critical pore size > 100 micrometer that can ensure cell migration ,fluid flow, bone ingrowth and vascularization ; optimum sterilizability for clinical applications,appropriate bonding to the host tissue without any scar formation, similar resorbation as the tissue growth rate, high osteo-compatibility and larger surface coverage by bone in growth and it must be up-scalable for mass production.

From Ref [29], In addition to providing a 3-D scaffold to permit bone bridging to occur, the ions released from bio glass played an imperative role in osteo-stimulation in-situ. Release of these ions at an optimum rate is required to allow cell signaling and recruitment for consequent bone formation in-vivo. In general bio glasses made by sol-gel method release high concentration of calcium ions into body fluid. This escalates the pH which is not accommodating to cell attachment and growth. Prior in-vitro studies revealed that these scaffolds release a large amount of calcium in the first three days of contact with tissue culture medium followed by a steady state release henceforth. Thus scaffolds which were preconditioned for three days displayed better extensive matrix formation as the initial surge of calcium release is eradicated resulting in a slower and consistent release rate of ions.70S30C scaffolds (70% SiO_2, 30%CaO) were produced by a sol-gel foaming process. Among dry, wetted and pre-conditioned 70S30C scaffolds, only the preconditioned sample displayed above 60% bone contact which was quite identical to that of NovaBone (melt derived 45S5 Bioglass) and Actifuse (Porous silicon substituted HA of 0.8

weight %).This result suggested that bio active glasses should be re-designed to ensure the usage of sol-gel scaffolds without preconditioning avoiding excess calcium release [29].

From [28], Possibly the most promising 45S5 Bio glass -containing composites which are essential for bone regeneration are thermally induced phase separated foams to form bio-degradable polymer composite with enhanced bioactivity [25]. The polymer is made to dissolve in dimethyl carbonate and then the glass fraction is added. This is followed by liquid nitrogen quenching and subsequent storage at 10 ^{o}C. In the end the lyophilization of the solvent takes place. PDLLA foams containing 40 wt. % Bio glasses 45S5 were produced with 97% porosity. Composite scaffolds are porous bone-like structures which permit the migration of oxygen and nutrients. Bio glass surface was improved by contact through solutions and interaction of polymeric matrix in scaffold fabrication .The polymer network reduced the Na^{+}/H^{+} exchange occurring in bio glass. Salt particles were leached from within the scaffolds for creation of pores with the aid of DI water [28]. Van der Waals interactions and hydrogen bonding likewise play an imperative role in the interactions and stability generation by compositing the polymer with the bioactive glass sol. Lately, hybrid organic-inorganic scaffolds have been prepared through the sol-gel process [17, 22–25], by hydrolysis of tetraethyl orthosilicate in the presence of an acid solution. Subsequently calcium chloride along with other metallic ions was added. PVA solution, surfactant, and hydrofluoric acid were also added systematically to the sol. Then the mixture was subjected to strongly agitation for formation of foam. The foam was put in containers for gelation followed by drying at low temperatures to elude thermal degradation of polymer. Low temperature is preferred as some unreacted species may give rise to cytotoxicity [24, 25].

As stated by *Bonnelye E, et al* [40], the SrO/CaO substitution has been found to demonstrate a linear growth of glass density along with a linear drop in oxygen density, resulting in less compact structures. It has been found that 0.1 weight% of strontium can lead to almost 15% increase in cell growth. With increase in Sr/Ca substitution the setting time of glass matrix decreases. This is due to the fact that strontium has higher basicity than calcium. Due to its high molecular weight; the integration of strontium results in actuated increase in silica content (in mole %), thereby increasing network connectivity. Strontium doped sol–gel glasses also depicted lower rates of dissolution.. However, there was an augment in the formation of HCA layer [24]. Strontium is perfect where bone metabolism is weak as it diminishes the dissolution of glass matrix by retarding the phenomenon of Si-O-Si bond breakage and silanol bond formation. Strontium plays a dual role in both promoting osteoblast proliferation and impeding bone resorption [28]. The addition of strontium ions in bio-glass solutions has earned great interest in bio glass applications owing to the fact that they support bone growth and augment bioactivity. Strontium ions constrain osteoclast activity for which they are used in the treatment of osteoporosis [40]. Nevertheless, excess inhibition of osteoclasts may thwart long-term regeneration of bone [41]. Ions used for stimulating osteogenesis are Li^{+},Zn^{2+},Sr^{2+},Mn^{2+} where as those used for enhancing angiogenesis are Mg^{2+},Cu^{+},Co^{+}.Both Cu^{+} and Co^{+} are cytotoxic as they cause oxidative damage to cells at higher concentrations. However B^{3+} ions are used to augment both osteogenesis and angiogenesis [41-48].

From [30], It was observed that degree of hydrolysis and concentration of polymer used in manufacturing bio glass PVA scaffolds played an important role in their properties. Considerably for higher % of PVA concentration, hydrofluoric acid desired for gelation purpose was comparatively higher. Owing to the high viscosity of the solution, the greater concentration of

hydroxyl groups contributes to cross-linking with the poly-siloxane network via hydrogen bonds and association with silanol groups.

From*Ref.* [49], we came to know that coatings in bioactive glasses are significant for metallic implants. Metals are bio-inert and are encapsulated within the fibrous tissue after the process of implantation. These coatings upsurge the implant stability by formation of bonds with the host bone. Besides excess bioactive coating will commence the instability of the metallic implants after a long duration of time. Thus their applications are limited to dental field .During the application of glass coatings, the thermal expansion coefficient of the glass must match with that of the metal to avoid pulling away of glass from the metal in the course of treating [49]. The thermal expansion coefficient of the original 45S5 composition does not match with that of the metals. In addition to that Bioglass 45S5 results in crystallization on sintering. In order to adjust TEC of the glass with respect to metal alloy, some amount of Na_2O and CaO is substituted with K_2O and MgO, respectively [49].

As per *Fu Q, et al* [44], the control of morphology of pores can be carried out more accurately via additive manufacturing techniques which involves the layer wise formation of scaffolds [44]. In these methods the pore structure of scaffolds is regulated by a computer-aided design. Additive manufacturing forms scaffold having high strength properties. This is because the pore channels are highly interconnected maintaining high alignment at comparatively low percentage porosity (50–60%). Various additive manufacturing techniques are Robocasting (3-D printing process): It produced scaffolds having >150 MPa compressive strength in the direction of the pore channels maintaining 60% porosity. The extrusion of inks was carried out via a syringe nozzle. Printing was done on an alumina substrate in non-wetting oil with the assistance of a

robotic deposition device .Subsequently drying was carried out followed by sintering at 700 ^{o}C [45, 46]. Freeze extrusion fabrication (FEF): It involves the mechanisms of both freeze–drying and extrusion printing. Scaffolds prepared by this method possessed 50% porosity along with compressive strength of 140 MPa [47, 48]. Extrusion of bioactive glass–polymer paste was carried out followed by its layer wise deposition in a cold atmosphere. In order to eliminate the water existing in the paste before performing sintering at 700 ^{o}C, freeze drying was implemented. Selective laser sintering (SLS): In this method laser is conveyed over a powder bed. Scaffolds yielded by this method showed 50% porosity with compressive strength of 20 MPa [50].

3. EXPERIMENTAL WORK

3.1. Manufacturing of Hybrid Samples : The manufacturing of the inorganic-organic hybrids was based on PVA based sol-gel-derived bioactive glass (BaG).The key parameters while selecting the PVA for this process were degree of hydrolysis and molecular weight keeping in mind its usage in biomedical applications. Deionized water was used in preparation of solutions. 25 weight% concentration PVA solutions were prepared by dissolution of PVA powder in a water bath, temperature being maintained at 80◦C with continual stirring for a period of 2 hours. 2N HCL solution (4-5 drops) was added in order to maintain 2.0 ± 0.1pH accordingly.

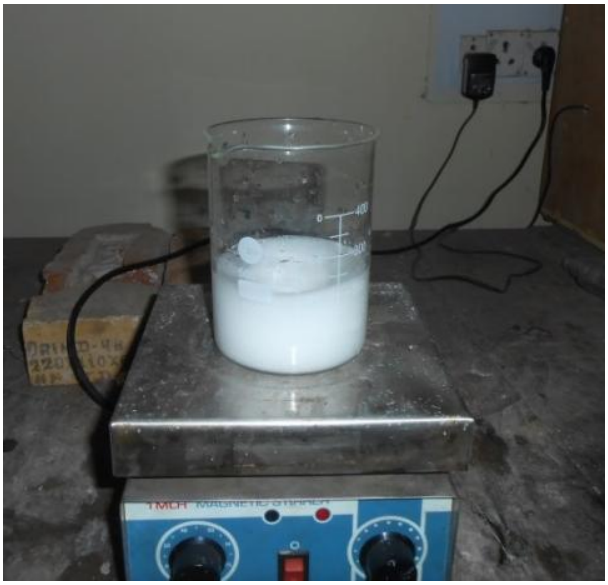


Fig (2): Sol gel hybrid formation by foaming.

3.1.1. Preparation of the Bioactive Glass Solution.

Two different bio glass compositions were prepared: first contained Strontium and second had no stontium. The two bioglass were created by mixing tetraethoxysilane (TEOS), DI water, triethylphosphate (TEP), calcium chloride and strontium chloride. First the solution was acidified with 3- 4 drops of hydrofluoric acid 2N solution. The hydrolysis of TEOS (glass former unit)

was carried out in acidic solution with continuous stirring. as illustrated in Figure 1. The H_2O: TEOS molar ratio was kept at 10: 1. For adding CaO and SrO, their respective chlorides were used instead of their nitrates as they would decompose at high temperatures. Batch calculations were carried out for 5 grams of batch maintaining composition of 30 wt% glass and 70 wt% polymer (PVA solution). While calculating the amount of TEOS and TEP required for each composition, their purity was taken into account.

Fig (1): Formation of bio glass solution.

Table (3) : Composition for bioglass scaffolds.

Composition	**SiO_2**	**P_2O_5**	**CaO**	**SrO**
1	65	5	30	0
2	65	5	15	15

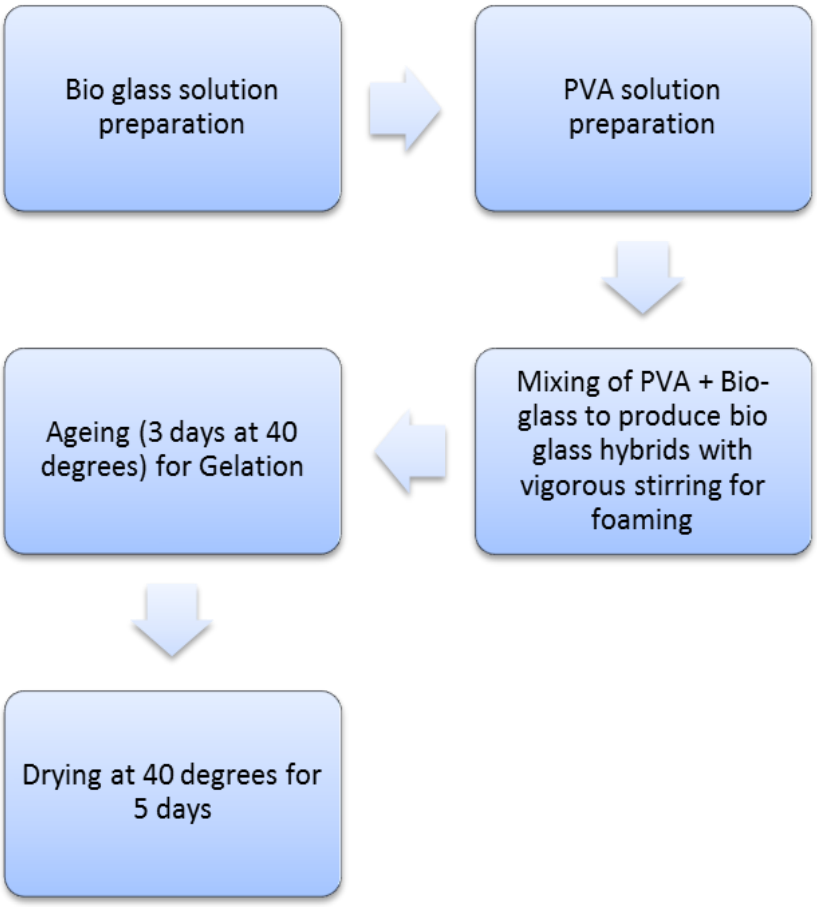


Figure (3): Flowchart of experimental work.

3.1.2. Preparation of the PVA-Bioactive Glass Hybrid Foams.

The composition of the prepared hybrid involved 30wt% glass and 70wt% polymer. Requisite amount of glass precursors was added to the PVA solution and stirring was carried out for five minutes. The surfactant, **TRITON-X100** was used as an agent to ensure foaming, and HF 40% v/v solution was added to the resulting solution for the purpose of gelation. Vigorous agitation of

the mixture was carried out as depicted in Fig (2). The foams were cast in plastic containers after gelation had occurred. The containers were sealed to make them air tight. The ageing of samples was carried out over a period of for 2-3 days at 40◦C. Then drying was done for a period of 4-5 days at the same temperature maintained during ageing. Low temperature was favored in order to avoid polymer thermal degradation. These hybrids formed after the drying process was PVA/BaG samples as depicted in Fig (4).

Figure (4): Top view of Sol gel hybrid scaffolds after ageing and drying.

3.2. Crystallinity and Phase Characterization by X-Ray Diffraction.

X-ray diffraction (XRD) patterns of hybrid PVA BaG hybrid was carried out using CuKα radiation having λ = 1.5 °A. XRD analyses were performed in the 2θ range from 15 to 90◦. The identification of major peaks was done using the "*Joint Committee on Powder Diffraction Standards.*"

3.3. Scanning Electron Microscopy (SEM).

In order to gauge the morphology of pores along with their size distribution, SEM images of organic-inorganic hybrids were captured using Nova nanoscan 450 microscope. The capturing of images of secondary electrons was done using an accelerating voltage of 8- 14 kV. The analysis of distribution of pore size distribution analysis was carried out utilizing the image analysis software.

3.4. Degradation Behavior—In Vitro Assay.

Mass loss measurements are essential in order to assess the degradation nature of scaffolds. The effect of CaO/SrO substitution on the degradation performance of the hybrid foams was carried out by assessing the mass loss of samples .The samples were kept in DI water for different periods of time. Three samples for each composition and time periods were cut as depicted in Figure (4). They were then weighed. It was then followed by the retaining of samples in a desiccator containing silica gel for a period of 24 hours maintaining vacuum conditions. A temperature of 25 ± 5◦C was maintained. The weighing of samples was done once more, and this was repeated until the equilibrium was attained as depicted in Figure (5).

Figure (5): Top-view of cut samples for biodegradation tests

The samples were immersed in DI water and maintained for periods of 3, 6, 9 and 12 days as shown in Figure (6) .Subsequently they were again dried under vacuum and weighed until equillibrium was attained.

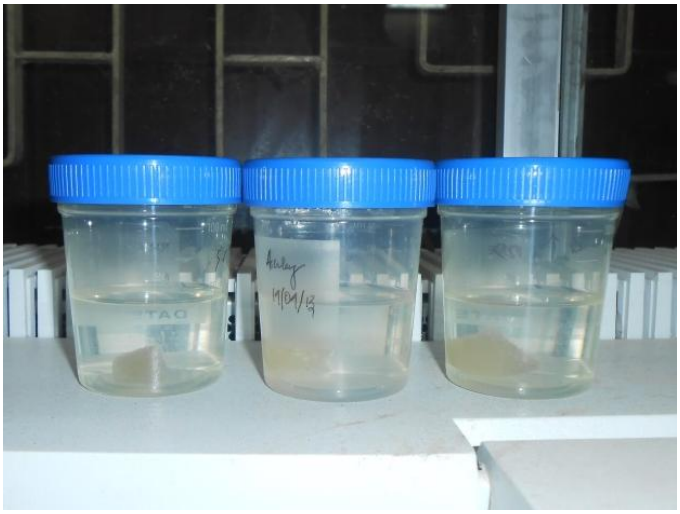


Figure (6): Side view of samples immersed in solution for evaluating biodegradation behavior.

The calculation of mass loss was carried out in accordance with the equation:

$$\Delta M = (M_{Si} - M_{Sf})/M_{Si} \times 100\% \quad (1)$$

Here M_{Si} is the dried weight before the process of immersion and M_{Sf} is the dried weight after the process of immersion for a given period of time in the degradation medium.

Figure (7): Top view of dried samples after 8 days of degradation.

XRD and SEM of final sample were carried out after 8 days of treatment with DI water. The final samples after their treatment over a period of 8 days are shown in the above figure (7).

3.5. In vitro bioactivity test

The valuation of in vitro bioactivity was done in a SBF (stimulated body fluid) solution.The composition of SBF solution is analogous to that of the human plasma .It was prepared by the dissolution of NaCl, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$ and Na_2SO_4 in redistilled water maintaining buffer at pH 7.4 with the assistance of tris(hydroxymethyl) amminomethane$(HOCH_2)3CNH_2$ and HCl. Each specimen was submerged in 30 ml of SBF solution in a polyethylene bottle covered with lid tightly as depicted in Figure (8). The bottles were stored in an incubator maintaining temperature of 40 °C for gaps of 1, 7 and 14 days. After soaking was carried out, the collection of samples was done. They were then subjected to treatment with distilled water and desiccation was carried out. SEM, XRD and FTIR measurements were carried in order to conclude the growth of the hydroxyapatite layer.

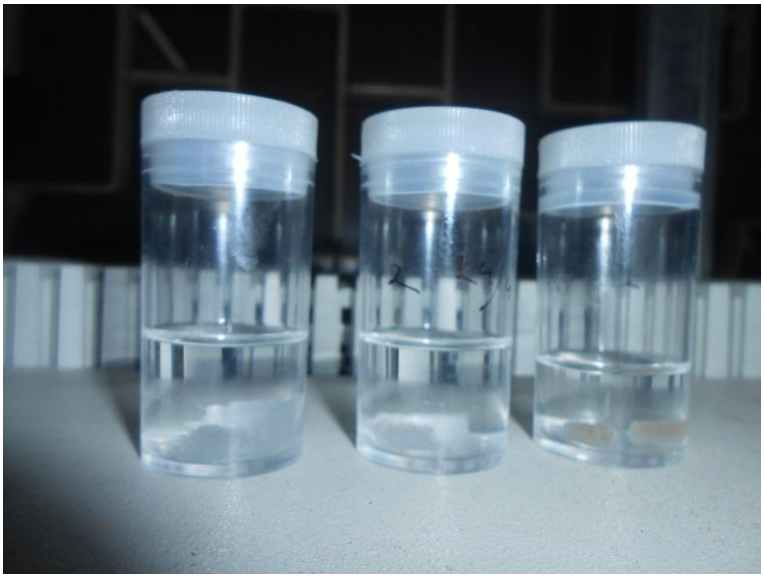


Figure (8): Side view of samples immersed in SBF for evaluating bioactivity performance.

4. Results and Discussion

4.1. Porosity measurement.

Table (4): AP and BD measurement of bioglass scaffolds.

Composition	Dry weight (in grams)	Suspended weight (in grams)	Soaked weight (in grams)	Bulk density (in gm/cc)	Apparent porosity (in %)
1(Ca only)	4.615	1.91	5.837	0.94	34
2(Ca + Sr)	4.82	1.83	5.982	0.92	36

Both the samples have about 35 % porosity. Usually, bio glass scaffolds have high porosity of > 60 %.The comparative lower porosity of the samples can be attributed to ineffective foam stabilization and comparatively low degree of hydrolysis of PVA used.

4.2. Characterization of Hybrids by XRD analysis.

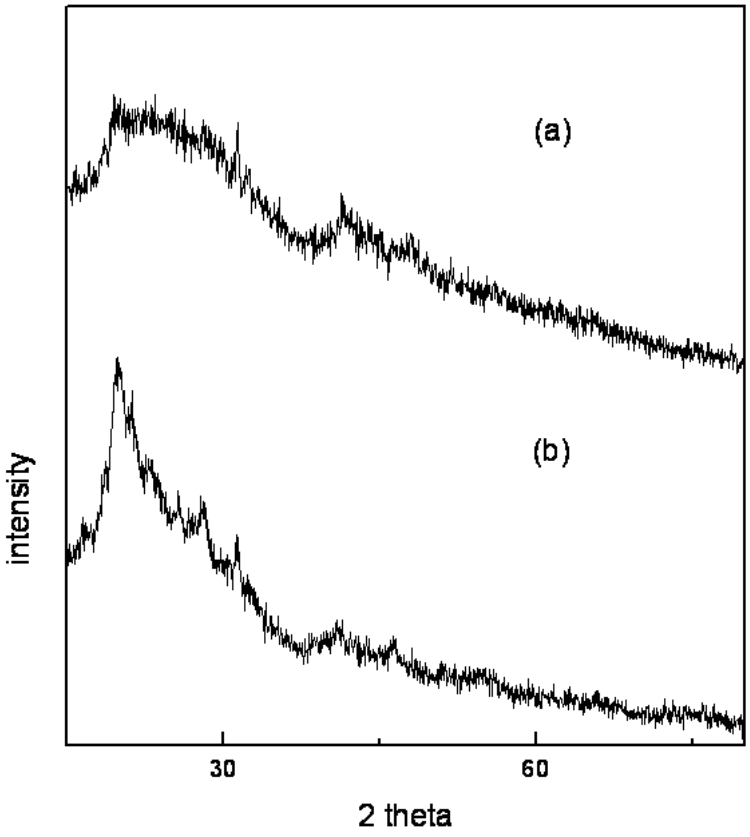


Figure (9): XRD phase identification of bio glass hybrid scaffolds.

In Figure (9),(a) and (b) represent the XRD patterns of composition (2) and (1) respectively. Composition (1) has only calcium whereas composition (2) has both calcium and strontium. PVA / BaG Hybrid mostly depict an amorphous structure as no specific high intensity peaks were found. It is mostly affected by synergetic effect of BaG and PVA. A significant peak was observed for (Ca + Sr) composition. This may be due to the occurrence of characteristic peak of 100 % pure crystalline silica at 20 degrees (2 theta)

4.3. Scanning Electron Microscopy (SEM).

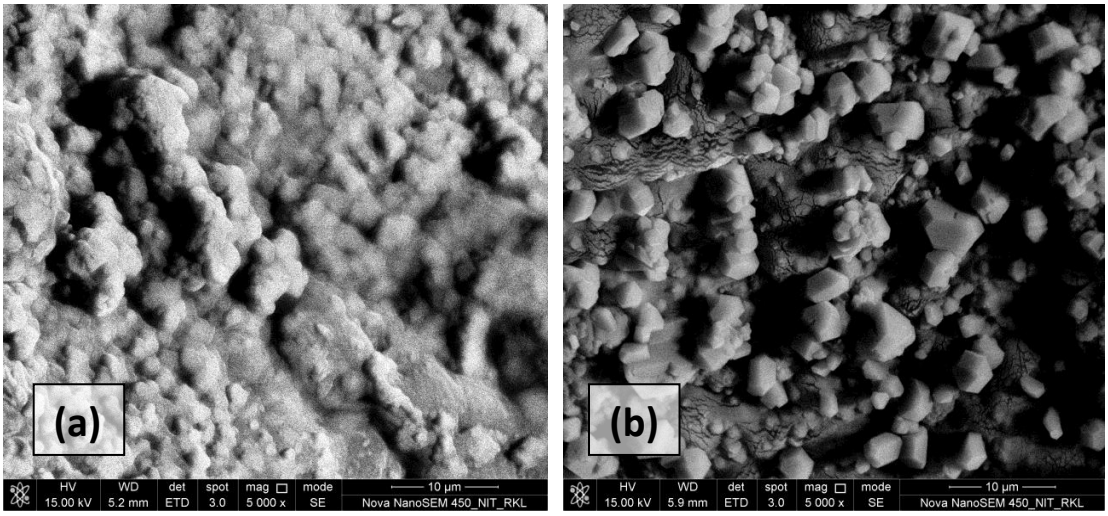


Figure (10): FESEM micrograph of as prepared sample of (a) Ca and (b) Ca+Sr based glass ceramic- PVA hybrid scaffold

The above figure (10) b and c depict the SEM images of the original sol-gel hybrid glass (untreated) at higher magnification.

4.4. Biodegradation behavior analysis.

Four different samples were cut each for composition 1 and 2 and were subjected to biodegradation test by immersing it in distill water and tris buffer solution. Composition 1 contained calcium whereas Composition 2 contained both Sr and Ca.

Table (5): Biodegradation behavior (with distill water).

Treatment Time (in days)	Composition	Original weight (in grams)	Reduced weight (in grams)	% mass loss
3	1	0.853	0.723	15.24
6	1	0.526	0.400	23.95
9	1	0.920	0.690	25
12	1	0.551	0.411	25.41
3	2	0.576	0.472	18.05
6	2	0.897	0.680	24.19
9	2	0.775	0.573	26.06
12	2	0.918	0.674	26.58

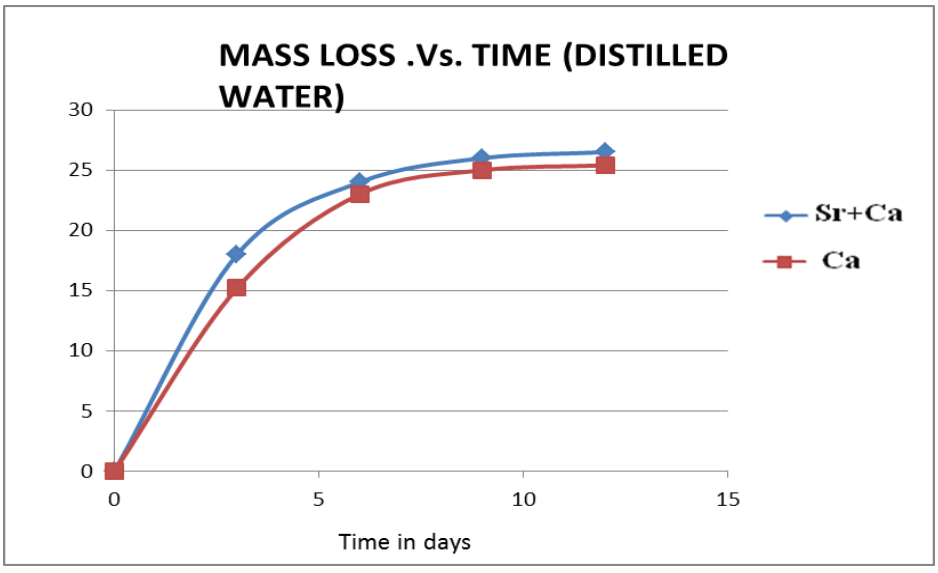


Figure (11): Mass loss % vs time curve (biodegradation in distill water)

Table (6) : Biodegradation behavior (with tris buffer).

Treatment Time (in days)	Composition (in grams)	Original weight (in grams)	Reduced weight (in grams)	% mass loss
3	1	0.88	0.72	18
6	1	0.74	0.54	27
9	1	0.802	0.56	30
12	1	0.665	0.46	31
3	2	0.87	0.73	16.25
6	2	0.65	0.48	25.6
9	2	0.68	0.482	29
12	2	0.93	0.65	30

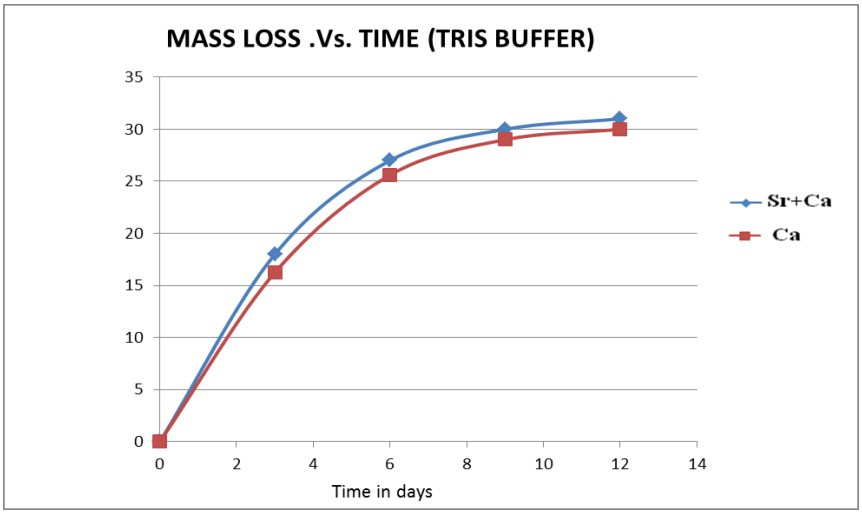


Figure (12): Mass loss % vs time curve (biodegradation in tris buffer solution).

From Figure (11) and (12), it can be clearly inferred that in both cases of distill water and tris-buffer solution; Strontium based samples showed comparatively higher degradation rates. This can be attributed to the osteoinductive properties of strontium.

4.5. Chemical Characterization by FTIR Spectroscopy.

Fourier transform infrared spectroscopy (FTIR) was utilized to characterize the existence of specific chemical groups present in the PVA/BaG hybrid samples. These spectra were meticulously used as reference for the effect of all the components on the produced hybrid network.

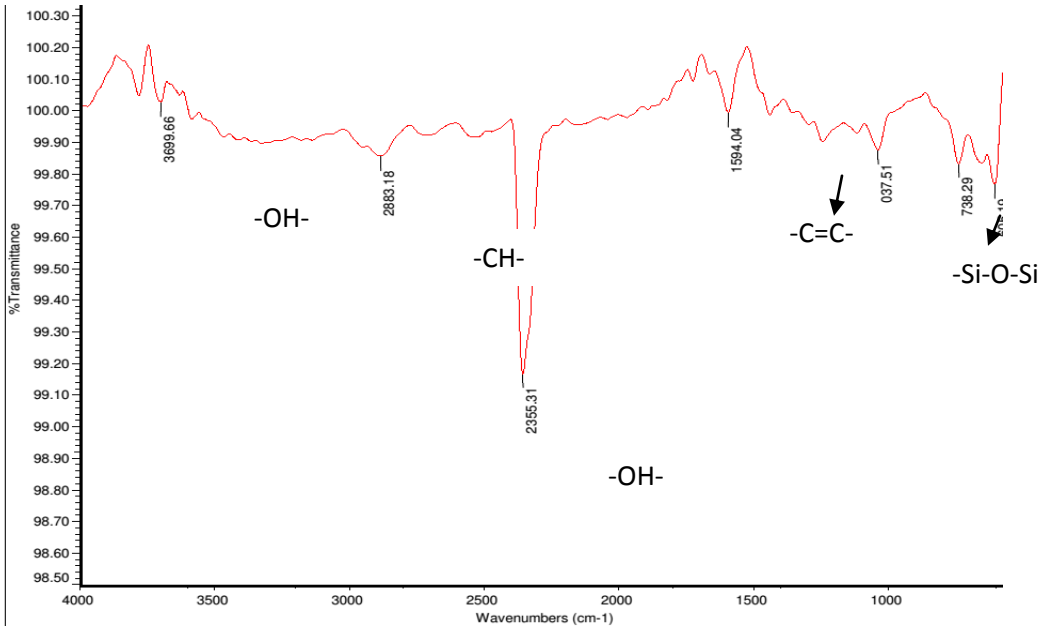


Figure 13(a): % Transmittance .Vs. Wave number (cm^{-1}) (FTIR spectroscopy)

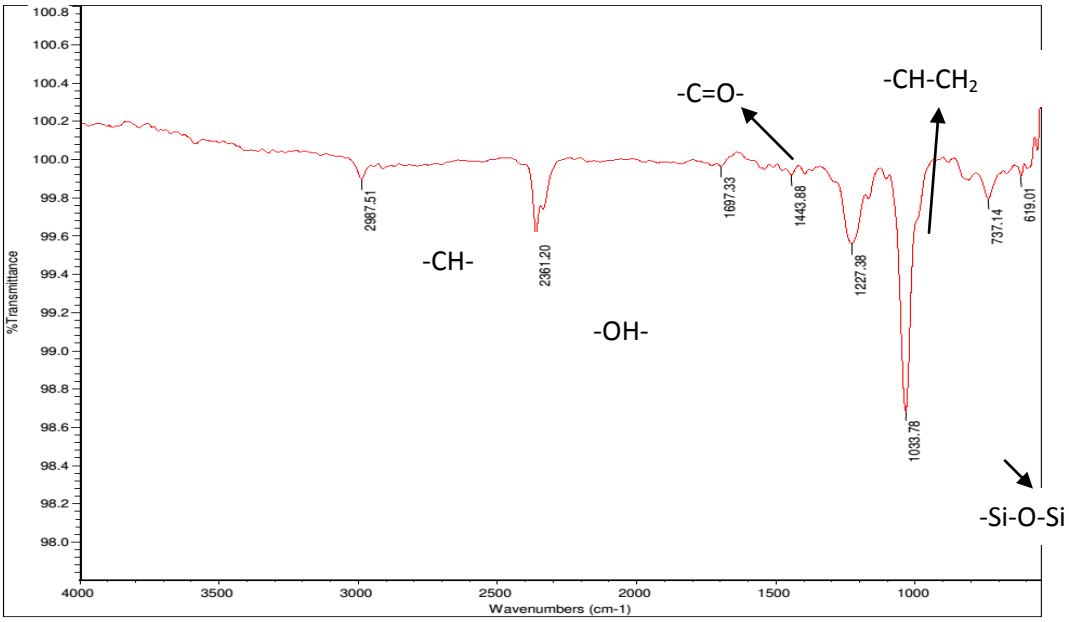


Figure 13 (b): % Transmittance .Vs. Wave number (cm^{-1}) (FTIR spectroscopy)

Figure (13) a and b illustrate the FTIR analysis of compositions having only Ca and both (Ca + Sr) respectively.

FTIR spectra was attained in the range 600-4000 cm^{-1}.Then spectra was utilized for reference to assess the contribution of all components of the hybrid network.

The broad band observed from 2350 to 2370 cm^{-1} and3600 to3700 cm^{-1} was found to belong to the hydroxyl(-OH-)groups stretching owing due to significant intramolecular as well as intermolecular hydrogen bonds. The band at 2870 to 2910 cm^{-1} was found to belong to the alkyl groups stretching mode (-CH-).The range of 1700 to1750 cm^{-1} absorption bands was owing to the stretching vibration of C=0 and C-O of remaining vinyl acetate on hydrolyzed groups of PVA used in making hybrids .The range of 1590-1610 cm^{-1} adsorption bands showed C=C stretching vibration.

Some other peaks were found related to PVA .The region 1435-1600 cm^{-1} were assigned to (CH)-(CH_2) vibrations whereas 610-620 cm^{-1} belonged to alkyl chain vibrations. Moreover, the region 1110-1130 cm^{-1} was identified as stretching up of siloxane bonds (Si-O-Si).

4.6. In vitro bioactivity test:

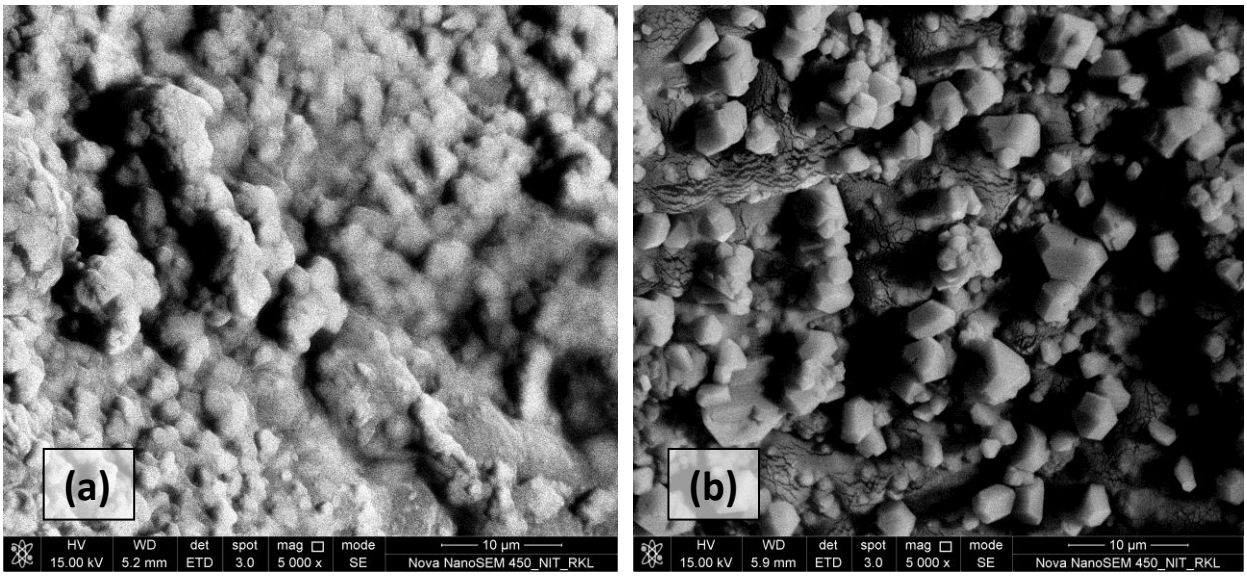


Figure 10 : FESEM micrograph of as prepared sample of (a) Ca and (b) Ca+Sr based glass ceramic- PVA hybrid scaffold

The micrograph shows that there is crystallization of glass ceramics in PVA matrix.

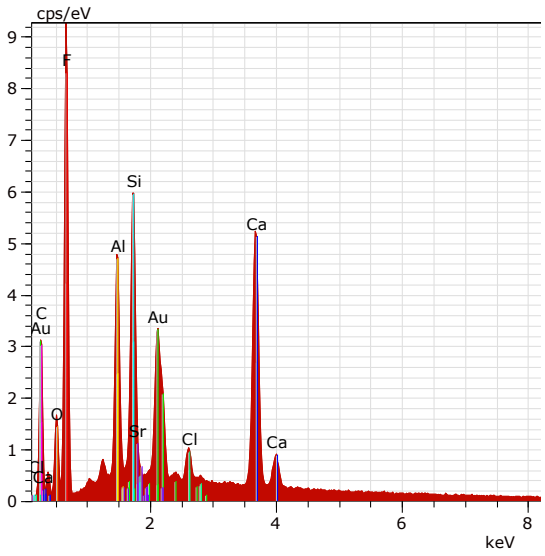


Figure 14 : EDAX Analysis of as prepared sample of Ca+Sr based glass ceramic- PVA hybrid scaffold

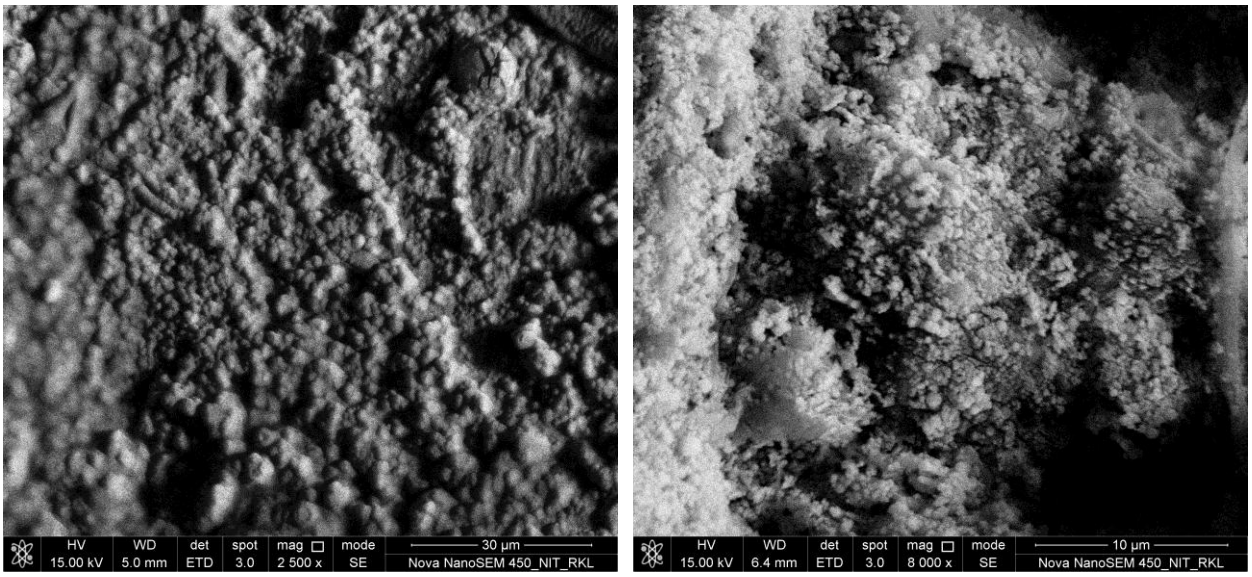


Figure 15: FESEM micrograph of Ca based glass ceramic- PVA hybrid scaffold after 14 days immersion in SBF.

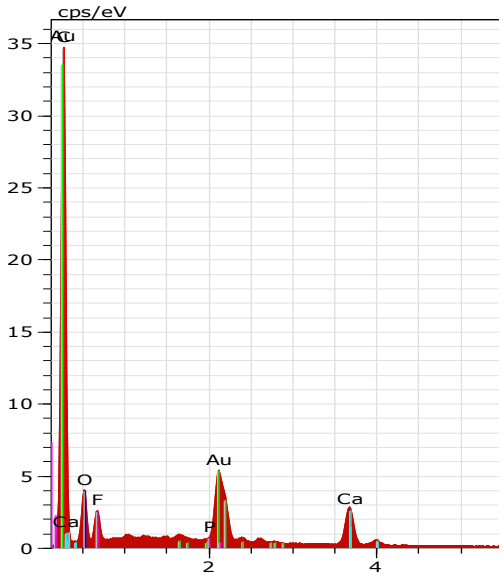
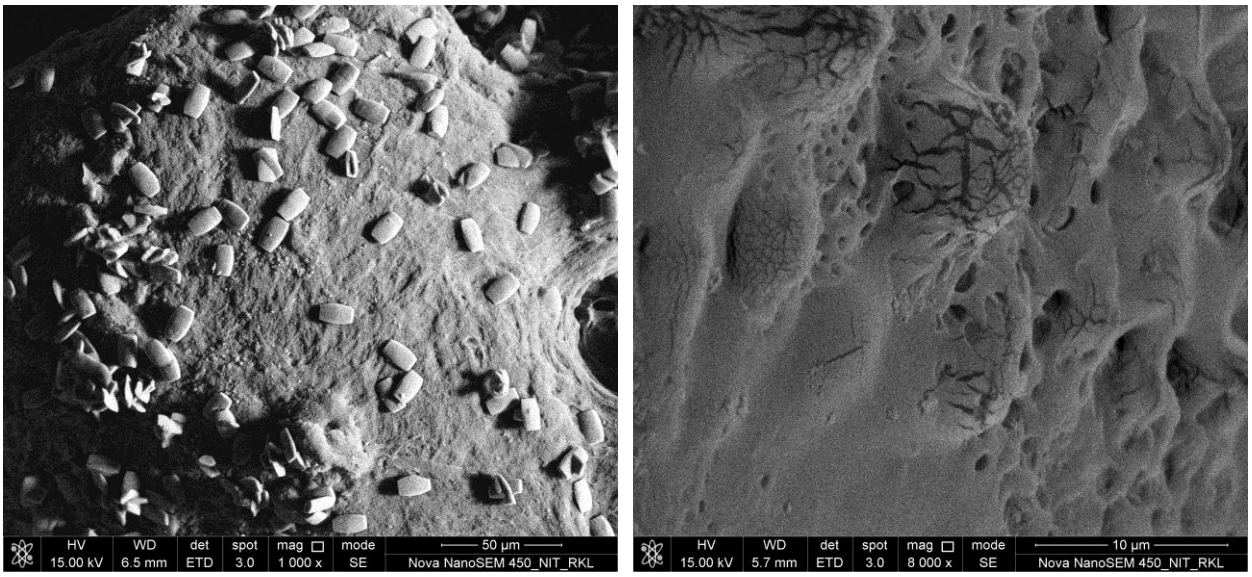


Figure 16: EDAX analysis of Ca based glass ceramic- PVA hybrid scaffold after 14 days immersion in SBF.



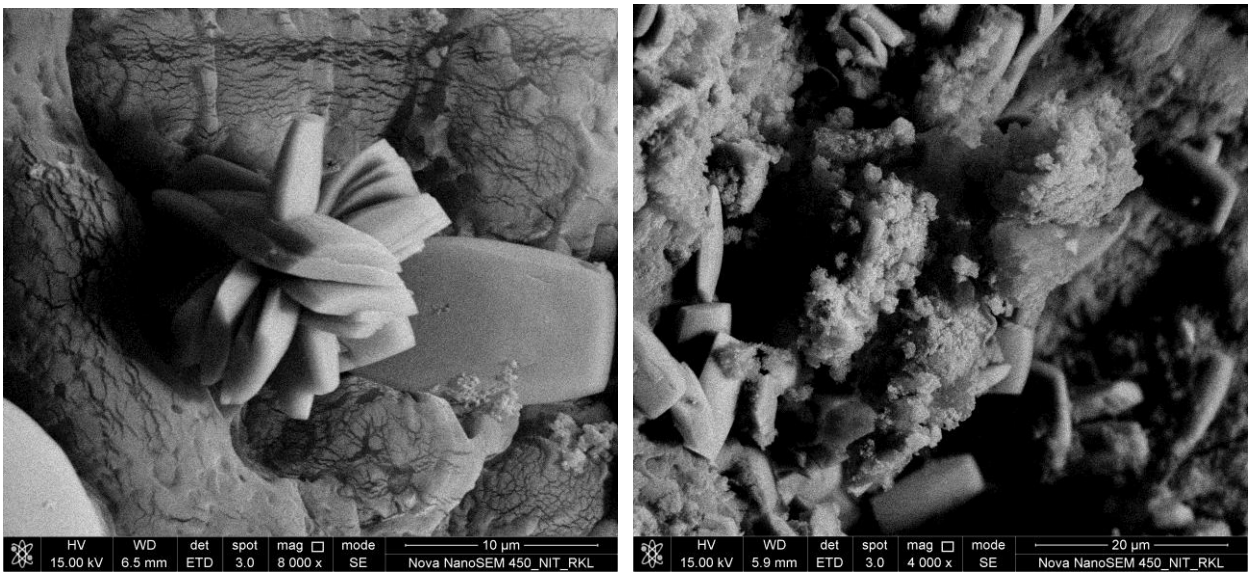


Figure 17: FESEM micrograph of Ca +Sr based glass ceramic- PVA hybrid scaffold after 14 days immersion in SBF.

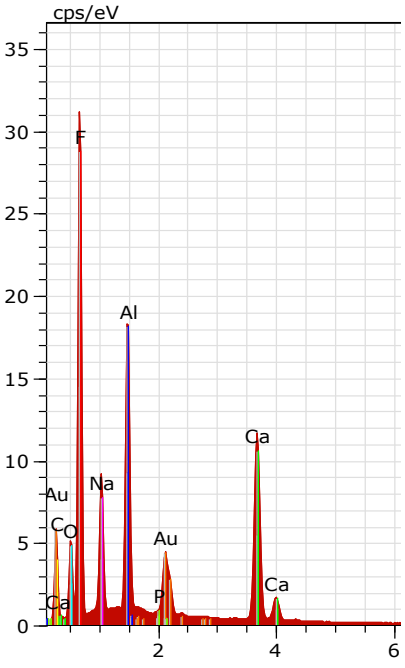


Figure 18: EDAX analysis of Ca+Sr based glass ceramic- PVA hybrid scaffold after 14 days immersion in SBF.

5. Conclusion

A hybrid scaffold based on 70 % polyvinyl alcohol and 30 % bioactive glass ceramics was synthesized and characterized for its biocompatibility and its degradation behavior. The glass ceramic was based on 65 % SiO_2, 5 % P_2O_5 and 30 % CaO. The porosity attained in the samples was about 35 % with bulk density of about 0.9 g/cc. XRD pattern showed the hybrid to be an amorphous material. The scaffold shows a degradation of about 30 wt% in 7 days. In case of Sr based scaffolds, FESEM studies showed a deposition of Na containing salt crystals on scaffold surface. Strontium based bio glass hybrids showed better bio-degradation rates than those of Calcium based only both in distilled water and tris-buffer solution. This may be accounted for the superior strontium osteointegration. This indicates that the hybrid scaffold is biocompatible and can have usage in bone tissue applications.

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